# Serum Response Factor (SRF)-Dependent Signaling in Regenerating, Hypertrophied, and Pahological Skeletal Muscle

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***Abstract***

Serum response factor (SRF) is an ubiquitously expressed member of the MADS (MCM1, Agamous, Deficiens, SRF) box transcription factor family, sharing a highly conserved DNA-binding/dimerization domain, which binds the core sequence of SRE/CArG boxes [CC (A/T)6 GG]. In addition, SRF is as well a widely expressed transcription factor involved in orchestrating disparate programs of gene expression linked to differentiation and cellular growth in smooth, cardiac, and skeletal muscle. Recent results obtained with specific SRF knock-out models, using the Cre-LopP system, emphasize a crucial role for SRF in postnatal skeletal muscle growth and regeneration possibly due to the direct binding of Interleukin-4 (IL-4) and Insulin-like growth factor (IGF-I) promoters in vivo. SRF also enhances the hypertrophic process in muscle fibers after mechnical overloading. During muscle hypertrophy, SRF seems to promote the proliferation and differentiation of muscle stem satellite cells after the activation of upstream mediators (STARS, MRTF-A, and RhoA). Our recent study has demonstrated a marked decrease in the amounts of SRF, STARS, and MRTF-A in sarcopenic muscles of mice. A decrease of SRF expression achieved using a transgenic approach was found to accelerate the atrophic process in muscle fibers and result in the significant deposition of intermuscle lipid with aging. A number of experimental models and human disorders are associated with changes in SRF expression, suggesting that SRF has a role in the pathogenesis of disease. This review summarizes the functional role of SRF and SRF-linked molecules during myogenesis, postnatal growth, hypertrophy, regeneration, and muscle wasting (ex. sarcopenia). Controlling the amount of SRF may be effective in the treatment of muscular disorders.

***Keywords***

*Skeletal Muscle, Serum Response Factor, Myod, Hypertrophy, Regeneration*

## Introduction

SRF is an ubiquitously expressed member of the MADS (MCM1, Agamous, Deficiens, SRF) box transcription factor family, sharing a highly conserved DNA-binding/dimerization domain, which binds the core sequence of SRE/CArG boxes [CC (A/T)6 GG] as homodimers [Treisman, 1987]. The DNA-binding properties and molecular cloning of SRF were first defined in the laboratory of Richard Treisman [Treisman, 1987]. Functional CArG boxes have been found in the cis-regulatory regions of various muscle-specific genes, such as the skeletal $\alpha$-actin, muscle creatine kinase, dystrophin, tropomyosin, and myosin light chain 1/3 genes. SRF-dependent signaling plays a major role in a variety of physiological processes, including cell growth, migration, and cytoskeletal organization [Teg Pipes et al., 2006]. For example, functional abrogation of SRF at embryogenesis led to a marked defect in the formation of smooth, cardiac, and skeletal muscle cells [Niu et al., 2005]. A dominant negative SRF mutant blocked the differentiation of coronary smooth muscle cells (SMCs) in the proepicardial organ of chick embryos [Landerholm et al., 1999] and disrupted skeletal and cardiac muscle differentiation in transgenic mice [Zhang et al., 2001].

Although the upstream modulators and co-activator or repressor for SRF are not completely clear, several candidates have been proposed. It is possible that the transcriptional activity of SRF is regulated by MuRF2 [Lange et al., 2005] and striated muscle activators of Rho signaling (STARS) [Kuwahara et al., 2005, Arai et al., 2002]. Lange et al. [2005] indicated that the RING/B-box dominates MuRF2 trap and abrogates SRF activity depending on the titin-Nbr1-p62/SQSTM1 signaling. Nuclear translocation of MuRF2 caused by mechanical inactivity leads to a great reduction in nuclear SRF [Lange et al., 2005]. In contrast, STARS contributes to the nuclear translocation of MRTF-A and MRTF-B [Kuwahara et al., 2005, Kuwahara et al.,

2007], and then these factors activate SRF transcription. Therefore, STARS, MRTF-A, and MRTF-B are positive upstream regulators for SRF-dependent signaling in skeletal muscle.

This review aims to outline the characteristics of SRF-dependent signaling and this functional role in hypertrophy, regeneration, and muscle wasting.

## Co-Activator and Repressor of Srf

Epc1 was first described to enhance the phenotypes of homozygotic mutations of the polycomb group gene in Drosophila. More recently, direct interaction between enhancer of Polycomb1 and SRF has been shown during skeletal muscle differentiation. Kim et al [2009] found that the Epc1-SRF complex bound to SRE, and the knockdown of Epc1 resulted in a decrease in the binding of SRF to the skeletal $\alpha$-actin promoter. In addition, their IP analysis indicated the direct binding of Epc1 to p300 in myoblast cells. Given these findings, Epc1 seems to be required for skeletal muscle differentiation, recruiting both SRF and p300 to the SRE of muscle-specific gene promoters.

Using the Xenopus, Meadows et al. [2008] showed that MASTR promoted the expression of skeletal muscle-specific genes by co-operating with SRF. Different from myocardin, the Xenopus MASTR activated the transcription of $\alpha$-tropomyosin and, to a lesser extent, $\alpha$MHC, skeletal $\alpha$-actin, and fast skeletal troponin I, in embryonic ectoderm explants. The muscle-marker activation by MASTR depended on the ability to interact with SRF, because a mutant form of MASTR lacking the SRF-binding domain failed to induce expression [Meadows et al., 2008]. The mouse MASTR protein lacks SRF-interacting regions, and so its assembly into a transcription regulatory complex may rely on interactions with other factors (e.g., MEF2 proteins). Intriguingly, mouse MASTR cooperated with MEF2 family proteins to stimulate transcription from MEF2 reporter plasmids in COS cells [Creemers et al., 2006].

MicroRNAs (miRNA) are a class of regulatory RNAs of ~22 nucleotides that post-transcriptionally regulate gene expression. miR-1 and miR-133 have distinct roles in modulating skeletal muscle proliferation and differentiation in cultured myoblasts in vitro and in Xenopus laevis embryos in vivo [Chen et al., 2006]. miR-133 may be a major upstream regulator for SRF in myogenesis. Indeed, treatment with double-stranded miR-133 significantly down-regulated the mRNA and protein expression of several differentiation-enhancing molecules (MyoD, myogenin, $\alpha$-actin, and MEF2D) in C2C12 myoblasts. The direct binding of SRF 3'UTR by miR-133 would inhibit the proliferation-repressing role played by SRF.

## Muscle Differentiation and Postnatal Growth

Conventional SRF knockout mice exhibit early embryonic lethality. To better understand the functional role of SRF in myogenesis, Li et al. [2005] created Myo-Cre mice using skeletal muscle-specific transgenes encoding Cre recombinase. The breeding of Cre transgenic mice heterozygous for the floxed Srf allele (Cre:SRF$^{flex1/+}$) with SRF$^{flex1/flex1}$ mice yielded Cre:SRF$^{flex1/flex1}$ mice. A histological analysis of skeletal muscle at E19.5 or birth from Myo-Cre; SRF$^{flex1/flex1}$ mice lacking skeletal muscle expression of Srf (70% decrease) showed the presence of multinucleated muscle fibers thinner than normal. A semiquantitative RT-PCR analysis of hindlimb muscle of Myo-Cre; SRF$^{flex1/flex1}$ mice showed a reduction in both skeletal and cardiac $\alpha$-actin mRNA (30%). Perinatal lethality is likely caused by abnormalities in the diaphragm muscle which prevent breathing. In addition, they created a MCK-Cre transgene to delete the SRF$^{flex1}$ allele and examine the role of SRF in muscle growth at a later time. SRF$^{flex1/flex1}$/MCK-Cre mice were mobile, nursed, and appeared normal at birth. However, by postnatal day (P) 3, these animals were lethargic and began to display growth retardation. A histological analysis of skeletal muscle from MCK-Cre/SRF$^{flex1/flex1}$ mutants at P3 showed thinner myofibers than normal, although the phenotype appeared less severe than that of Myo-Cre/ SRF$^{flex1/flex1}$ mutants.

Charvet et al. [2006] developed a conditional SRF gene inactivation strategy in the mouse, based on the Cre-LoxP system, which has been used to demonstrate that SRF is crucial for cardiomyogenesis and the maintenance of adult cardiac function [Parlakian et al., 2005]. Charvet et al. [2006] investigated the role of SRF in the postnatal development of skeletal muscles, using a HAS-Cre transgenic line in which Cre-mediated recombination occurs in postmitotic myofibers but not in satellite cells [Iyer et al., 2006]. Despite the death of 30% of mice lacking SRF in skeletal muscle fibers during the perinatal period, Charvet et al. [2006] was able to obtain and further analyze mutant mice. HAS-Cre:S$^{f}$/S$^{f}$ mice were indistinguishable from controls at birth but displayed growth retardation as early as day 3. Muscle lacking SRF had very low levels of muscle creatine kinase,

skeletal $\alpha$-actin, and beta-tropomyosin transcripts. They also observed a downregulation of IL-4 and IGF-I expression in mutant myofibers which could account for the defective growth.

## SRF and MRTF-A Expression in the Regenerating Muscle

Skeletal muscle satellite cells are generally in a quiescent state in adult muscle, but when minor damage or injury occurs, signals generated within the muscle activate these satellite cells, stimulating them to migrate to the site of damage where they proliferate, differentiate, and fuse with the damaged fibers or form new fibers [Hawke et al., 2001]. Studies in vitro have documented many factors, primarily protein growth factors, which can regulate satellite cell activity [Hawke et al., 2001]. In particular, insulin-like growth factor-I (IGF-I), whose expression is known to be upregulated in regenerating muscle in vivo, positively regulated the proliferation and differentiation of satellite cells/myoblasts in vitro.

Interestingly, HAS-Cre:$S^f/S^f$ mice exhibited defects in the regeneration of skeletal muscle after the injection of cardiotoxin, although the exact mechanism involved has not been elucidated. The SRF-depleted mice showed decreased levels of IGF-I and IL-4 mRNA at 2 months of age. Since mice with a downregulated IL-4 pathway regenerated normally [Horsley et al., 2003], Charvet et al. [2006] proposed that the regenerative defect was attributable to the decreased expression of IGF-I. During muscle regeneration, the defect of IGF-I expression may affect downstream molecules such as calcineurin [Semsarian et al., 1999]. Many studies have indicated defects in skeletal muscle regeneration when calcineurin was inhibited [Sakuma et al., 2003a; Sakuma et al., 2010a]. For example,it was showed that the inhibition of calcineurin by CsA induced extensive inflammation, marked fiber atrophy, the appearance of immature myotubes, and calcification in the regenerating muscle [Sakuma et al., 2003a]. Although Charvet et al. [2006] did not investigate whether HAS-Cre:$S^f/S^f$ mice had defective calcineurin signaling in these skeletal muscles, several downstream candidates of the signaling molecule, the nuclear factor of activated T cells c2 (NFATc2), MyoD, myogenin, and myostatin [Sakuma et al., 2010a], were modulated by the SRF mutation. By co-operating with transcription factors such as MEF2 and SRF, MyoD and myogenin have been shown to promote the transcription of many skeletal muscle-specific genes [Berkes et al., 2005]. Muscle regeneration in vivo is markedly impaired in MyoD null mice. The differentiation factor myogenin is necessary and sufficient for the formation of myotubes and fibers. Therefore, the impaired calcineurin-dependent signaling elicited by the reduction in IGF-I may regulate the regenerative defect recognized in HAS-Cre:$S^f/S^f$ mouse muscles.

More recently, Mokalled et al. [2012] demonstrated that members of the Myocardin family of transcriptional coactivators, MASTR and MRTF-A, are up-regulated in satellite cells in response to skeletal muscle injury. In addition, global and satellite cell-specific deletion of MASTR in mice impairs skeletal muscle regeneration. This impairment is substantially greater when MRTF-A is also deleted and is due to aberrant differentiation and excessive proliferation of satellite cells. In fact, double knockout satellite cells (MASTR and MRTF-A) exhibit a significant downregulation of various modulators of cell cycle arrest such as Retinoblastoma (Rb) and growth arrest and DNA damage-inducible 45a (Gadd45a).

MASTR appears not to function by binding directly with SRF. The mouse MASTR protein lacks SRF-interaction regions, and thus its assembly into a transcription regulatory complex would rely on interactions with other factors (e.g., MEF2 proteins). Mokalled et al. [2012] proposed the intriguing hypothesis that interaction between MASTR and MEF2 (MEF2A and MEF2C) enhances the expression of cell cycle arrest genes and MyoD expression at the transition from proliferation to differentiation in satellite cells. MASTR/MEF2 and MRTF-A/SRF complexes would therefore modulate MyoD expression during myogenic differentiation independently.

## Functional role of SRF in hypertrophied muscle

In adults, SRF activity could be important for the control of skeletal muscle mass. In fact, SRF also enhances the hypertrophic process in muscle fibers after mechanical overloading [Flück et al., 1999, Lamon et al., 2009, Sakuma et al., 2003b]. For example, Flück et al. [1999] utilized a stretch-induced hypertrophic model, in which a weight equal to 10% of body weight was attached to the left wing of a rooster to induce enlargement of the ALD muscle. They demonstrated an increase in SRF protein using both crude nuclear protein and cytoplasmic fractions in 7-day stretched ALD muscle. In humans, Lamon et al.

[2009] demonstrated that 8 weeks of resistance training (leg presses, squats, and leg extensions) induced increases in SRF mRNA (3-fold) and nuclear protein (1.25-fold) in the vastus lateralis muscle. In the same training period, they also observed a similar increase in the mRNA levels of several SRF-targeted molecules ($\alpha$-actin, MHC IIa, and IGF-I [Charvet et al., 2006]). They proposed the induction of these molecules by SRF in human hypertrophied muscle, though they did not provide any direct evidence. Although SRF would regulate proliferation and differentiation using different pathways, it would mainly activate the differentiation of satellite cells during muscle hypertrophy. Indeed, it was showed that, in mechanically overloaded muscles of rats, the SRF protein co-localized with MyoD and myogenin in myoblast-like cells during the active differentiation phase [Sakuma et al., 2003]. In this study, abundant SRF protein at 2 days was failed to be defected after mechanical overloading, when many proliferating satellite cells and/or myoblasts are expected to exist. In addition, the location of the SRF protein did not correspond with that of BrdU-positive satellite cells or ED1-positive macrophages in the hypertrophied plantaris muscle.

More recently, Guerci et al. [2012] investigated the functional role of SRF in adult mammalian muscle using $SRF^{flox/flox}$: HAS-Cre-$ER^{T2}$ mice injected withtamoxifen. During the compensatory hypertrophy phase, growth was completely slow in the SRF-deleted plantaris muscle, demonstrating that SRF is necessary for overload-induced myofiber hypertrophy. Intriguingly, Guerci et al. [2012] showed that the lack of SRF in myofibers affected satellite cell proliferation and fusion to the growing fibers. In their genetic mouse model, Cre recombinase is expressed only in myofibers but not in satellite cells. Furthermore, Guerci et al. [2012] identified the secreted molecules mediating these paracrine effects and whose expression is under the control of SRF by using a global transcriptomic approach allowing the identification of genes activated by SRF. In SRF-deleted muscles, the overexpression of IL-6 is sufficient to restore satellite cell proliferation, but not satellite cell fusion and overall growth. Cox2/IL-4 overexpression rescues satellite cell recruitment and muscle growth without affecting satellite cell proliferation, identifying altered fusion as the limiting cellular events precluding the hypertrophic growth of SRF-deleted muscles. Guerci's excellent finding was further supported by Bruusgaard et al., [2010], showing that the addition of nuclei precedes increased fiber size during compensatory hypertrophy and that

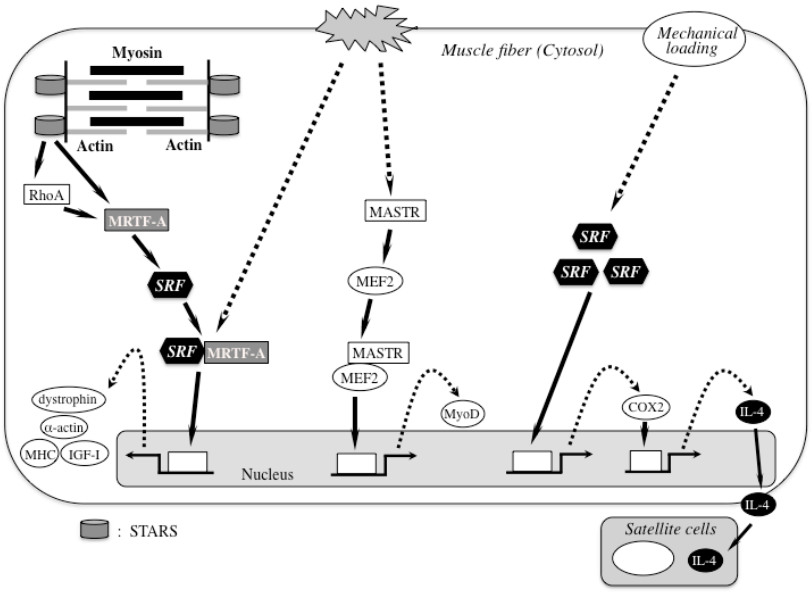


FIG. 1 SCHEMATIC DIAGRAM OF SRF-DEPENDENT SIGNALLING IN REGENERATION AND HYPERTROPHY OF SKELETAL MUSCLE. MECHANICAL LOADING PRODUCED BY MUSCLE CONTRACTION CAUSES MYOSIN AND ACTIN INTERACT , WHICH IN TURN ACTIVATES STARS. STARS PROTEIN ACTIVATES MRTF-A INDIRECTLY VIA RHOA OR DIRECTLY [KUWAHARA ET AL., 2005]. ACTIVATED MRTF-A BINDS TO SRF TO PROMOTE THE EXPRESSION OF MUSCLE-SPECIFIC GENES SUCH AS THOSE FOR $\alpha$-ACTIN, DYSTROPHIN, IGF-I, AND MYOSIN HEAVY CHAIN (MHC). IN THE DIFFERENTIATION PHASE OF MUSCLE REGENERATION, ACTIVATED MASTR BINDS TO MEF2 TO UPREGULATE THE EXPRESSION OF MYOD [MOKALLED ET AL., 2012]. IN MECHANICALLY OVERLOADED MUSCLE, SRF ENHANCES THE EXPRESSION OF COX2 mRNA, WHICH IN TURN UPREGULATES IL-4 mRNA, AND ULTIMATELY SECRETES IL-4 PROTEIN [GUERCI ET AL., 2012]. IL-4 PRODUCED BY MUSCLE FIBERS MOVES INTO SATELLITE CELLS PARACRINALLY TO MODULATE THE FUSION OF SATELLITE CELLS

this constituted the major cause of hypertrophy. However, the contribution of satellite cells to muscle hypertrophy has been a controversial issue [McCarthy et al., 2007, O'Connor et al., 2007]. In fact, McCarthy et al., [2011] suggested that satellite cell-depleted skeletal muscle undergoes extensive fiber hypertrophy after mechanical overloading. Therefore, further examination of SRF's role in muscle hypertrophy is needed. Figure 1 summarizes the functional role of SRF and SRF-linked molecule (MASTR) in regeneration and hypertrophy of skeletal muscle.

STARS, MRTF-A, and MRTF-B, upstream regulators of SRF, are also upregulated in the hypertrophied muscle. A real-time PCR analysis demonstrated that increased mechanical loading from resistance training in humans caused significant increases in the mRNA of STARS (3.4-fold), MRTF-A (2.5-fold), and MRTF-B (3.6-fold). More recent intriguing review [Wallace et al., 2012] has indicated a possibile modulation of proliferation and/or differentiation of satellite cells by STARS in skeletal muscle, although further extensive description would be needed in this molecule.

## Defects of SRF signaling with muscle wasting

### *Sarcopenia*

Aging is associated with progressive declines in muscle mass, quality, and strength, a condition known as sarcopenia. Lean muscle mass generally contributes to ~50% of total body weight in young adults, but this value declines with aging, to just 25% at 75-80 yr of age [Short et al., 2004]. At the muscle fiber level, sarcopenia is characterized with specific type II fiber atrophy and fiber loss [Larsson, 1978]. Although the specific contribution of each is unknown, several

possible signaling factors (Akt-mTOR, RhoA-SRF, and autophagy) have been proposed to contribute to age-related muscle atrophy [Sakuma et al., 2010b]. In fact, using crude and fractionated homogenates, our recent study has clearly demonstrated a blunted expression of SRF protein in the quadriceps and triceps brachii inactivated MASTR binds to MEF2 to upregulate the expression of MyoD [Mokalled et al., 2012]. In mechanically overloaded muscle, SRF enhances the expression of COX2 mRNA, which in turn upregulates IL-4 mRNA, and ultimately secretes IL-4 protein [Guerci et al., 2012]. IL-4 produced by muscle fibers moves intosatellite cells paracrinally to modulate the fusion of satellite cells. aged mice [Sakuma et al., 2008]. Immunofluorescence microscopy also indicated the selective down-regulation of SRF immunoreactivity in the cell cytosol but not in Pax7-labelled satellite cells in sarcopenic mice. In addition, our data showed a decrease in

MRTF-A mRNA (50-70%) and protein (76%) levels in only the nuclear fraction with age. Furthermore, 60 and 40 % decreases in the amount of STARS mRNA were observed in the quadriceps and triceps brachii of 24-month-old mice, respectively [Sakuma et al., 2008].

More recently, a decrease of SRF expression achieved using a transgenic approach was found to accelerate the atrophic process in muscle fibers with age [Lahoute et al., 2008]. These SRF KO mice showed marked deposition of intermuscle lipid with aging. One morphologic aspect of sarcopenia is the infiltration of muscle tissue components by lipids, because of the increased frequency of adipocyte or lipid deposition [Dube and Goodpaster, 2006] within muscle fibers. As with precursor cells in bone marrow, liver, and kidney, muscle satellite cells expressing the adipocytic phenotype increased with age [Shefer et al., 2006], although this process is still relatively poorly understood in terms of its extent and spatial distribution. Lipid deposition, often referred to as intra-myocellular lipid deposition, may result from a net buildup of lipids due to the reduced oxidative capacity of muscle fibers with aging [Dube and Goodpaster, 2006]. These lines of evidence clearly showed a defect of SRF-signaling in aged mammalian muscle.

### *Musclular disorder*

SRF appears to be linked to the degenerative process during muscular dystrophy. Significant reduction in the amount of SRF has been observed [Sakuma et al., 2004], 40-50% and 50-65% at 2 and 12 weeks of age, respectively, in merosin-deficient congenital muscular dystrophy. Our immunohistochemical analysis indicated that mature normal mice had an abundance of SRF protein in the cytoplasm of several muscle fibers, while the dy mice did not. In the skeletal muscle, there is no direct evidence of a link between SRF disorders and the pathogenesis of disease. However, Lange et al. [2005] observed that a mutation in the TK domain of titin, a possible upstream modulator of SRF, disrupted Nbr1 binding and led to hereditary myopathy with early respiratory failure (HMERF). HMERF patient biopsies revealed a diffusable localization of Nbr1, large cytoplasmic aggregates of p62, and the selective accumulation of

MuRF2 in centralized nuclei in diseased muscle. Unfortunately, their study did not examine the localization of SRF in the muscle of HMERF patients. In contrast, human heart failure was reported to show elevations of a natural dominant-negative form of SRF arising from alternative splicing [Davis et al., 2002]. The dominant negative SRF isoform potently inhibited SRF-dependent gene expression, mirroring the biochemical phenotype seen in SRF-null mice [Davis et al., 2002]. In addition, a subsequent human heart failure study showed decreases in full-length SRF and elevated expression of a caspase-3-cleaved product of SRF [Chang et al., 2003]. A more recent review [Miano et al., 2010] proposed various disorders to be linked with the SRF mutation as shown by many reliable studies using cell-specific SRF-knockout phenotypes.

## Conclusion

This review summarized and highlighted the currently understood role of SRF in the regulation of myogenesis, postnatal growth, hypertrophy, and the wasting of skeletal muscle. Intriguingly, our recent study showed that a defect of SRF-dependent signaling is involved in sarcopenia. Controlling the amount of SRF may be effective in the future treatment of muscular disorders.

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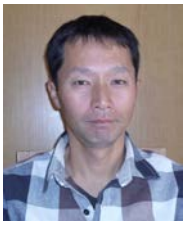

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April 1987-March 1991
Under graduate program, University of Tsukuba
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Doctoral program, University of Tsukuba
Type of Degree: PhD for Sports Sciences (University of Tsukuba, Tsukuba, Ibaraki, Japan, 1996)
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1996-2000 Researcher, Department of Physiology, Aichi Human Service Center

2000-2005 Assistant Professor, Department of Legal Medicine, Kyoto Prefectural University of Medicine

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Sakuma, Kunihiro, and Akihiko Yamaguchi. "Sarcopenia: Molecular mechanism and current therapeutic strategies." Cell Aging (NY, USA: Nova Science Publishers, 2011) : pp. 92-153.

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